CLAIMS

1. A method for screening substances which are potential inhibitors of transcription of bacterial T-box regulated genes, comprising the steps of:

a) incubating one or more assay mixtures comprising: a template DNA that comprises: (i) a bacterial promoter, (ii) a leader of a T-box regulated gene, including a transcription start site, and (iii) a downstream polynucleotide of sufficient length for detection of a read-through mRNA product; divalent metal cations; nucleoside triphosphates; dinucleotides corresponding to and encoded by the transcription start site of the leader; bacterial RNA polymerase complex; and tRNA specific for a specifier sequence located in the leader; and

b) incubating a potential inhibitor substance with one or more assay mixtures comprising: a template DNA that comprises: (i) a bacterial promoter, (ii) a leader of a T-box regulated gene, including a transcription start site, and (iii) a downstream polynucleotide of sufficient length for detection of a read-through mRNA product; divalent metal cations; nucleoside triphosphates; dinucleotides corresponding to and encoded by the transcription start site of the leader; bacterial RNA polymerase complex; and tRNA specific for a specifier sequence located in the leader; and

c) comparing the amount of the read-through mRNA product produced in step a) with the amount the read-through mRNA product produced in step b)

wherein a lesser amount of a read-through mRNA product determined for step b) in comparison with step a) indicates that said potential inhibitor substance inhibits transcription of said T-box regulated gene.

2. The method recited in claim 1 wherein the divalent metal cation is Mg^{2+}.

3. The method recited in claim 1 wherein the nucleoside triphosphates are selected from the group consisting of adenosine triphosphate, guanosine triphosphate, cytosine triphosphate, and uridine triphosphate, and any combination of one or more of these.

4. The method recited in claim 1 wherein the dinucleotides are selected from the group consisting of ApA, ApC, ApU, ApG, GpA, GpC, GpU, GpG, CpA, CpC, CpU, CpG, UpA, UpC, UpU, and UpG.

5. The method recited in claim 1 wherein the bacterial promoter is selected from the group consisting of the *B. subtilis glyQS* promoter and the *B. subtilis rpsD* promoter.

6. The method recited in claim 1 wherein the downstream polynucleotide of sufficient length for detection of a read-through mRNA product comprises a polynucleotide which is from about 30 to 150 nucleotide residues in length.

7. The method recited in claim 1 wherein the tRNA specific for a specifier sequence located in the leader is *B. subtilis* $tRNA^{Gly}$.

8. The method recited in claim 1 wherein the RNA polymerase is from either *B. subtilis* or *Escherichia coli.*

9. The method recited in claim 1 wherein the leader comprises a polynucleotide which is a variant of a wild-type glycine synthetase leader from a Gram positive bacterial strain, wherein the variant comprises modifications to one or both of the wild-type specifier and wild-type terminator sequences.

10. The method recited in claim 1 wherein the tRNA specific for a specifier sequence located in the leader is a variant of a wild-type tRNA wherein any one or more of the wild-type anticodon sequence, the wild-type discriminator sequence, and the transcription start site is altered to complement with the leader sequence.

11. The method recited in claim 1 wherein the leader comprises a polynucleotide which is a variant of a wild-type glycine synthetase leader sequence from a Gram positive bacterial strain, wherein the variant comprises modifications to one or both of the wild-type specifier and wild-type terminator sequences, and wherein the tRNA specific for a specifier sequence located in the wild-type leader is a variant of a wild-type tRNA wherein either or both of the wild-type anticodon sequence and the wild-type discriminator sequence are altered to complement with the variant leader sequence.

12. The method recited in claim 1 wherein the assay mixtures are *in vitro* halted-complex bacterial transcription assay systems.

13. A method for identifying inhibitors of transcription of bacterial T-box regulated genes, comprising:

providing two or more *in vitro* halted-complex bacterial transcription assay systems which comprise a template DNA comprising: (i) a bacterial promoter, (ii) a polynucleotide comprising a portion of a leader from the *B. subtilis glyQS* gene and includes a transcription start

site, and (iii) a downstream polynucleotide of sufficient length for detection of a read-through mRNA product; RNA polymerase, and uncharged *B.* subtilis $tRNA^{Gly}$, wherein at least one or more of said assay systems comprises a test substance, and wherein at least one or more of said assay systems lacks a test substance, and comparing the amount of *B. subtilis glyQS* read-through mRNA produced in each of said assay systems, wherein a test substance is considered an inhibitor if it effects a lesser amount of *B. subtilis glyQS* read-through mRNA produced in an assay system comprising such test substance as compared to an assay system lacking any test substance.

14. The method recited in claim 13 wherein the bacterial promoter is selected from the group consisting of the *B. subtilis glyQS* promoter and the *B. subtilis rpsD* promoter.

15. The method recited in claim 13 wherein the RNA polymerase is from either *B. subtilis* or *Escherichia coli.*

16. The method recited in claim 13 wherein the sequence of the polynucleotide comprising a portion of the *B. subtilis glyQS* leader is a variant of the wild-type *B. subtilis glyQS* leader sequence, wherein the variant comprises modifications to one or both of the wild-type specifier and wild-type antiterminator sequences.

17. The method recited in claim 13 wherein the uncharged *B.* subtilis $tRNA^{Gly}$ is a variant of a wild-type *B.* subtilis $tRNA^{Gly}$ wherein either or both the wild-type anticodon sequence and the wild-type discriminator sequence is altered to complement with the *B. subtilis glyQS* polynucleotide leader region.

18. An assay system for screening substances which are potential inhibitors of transcription of bacterial T-box regulated genes, comprising:

a) one or more assay mixtures comprising: a template DNA that comprises: (i) a bacterial promoter, (ii) a leader of a T-box regulated gene, including a transcription start site, and (iii) a downstream polynucleotide of sufficient length for detection of a read-through mRNA product; divalent metal cations; nucleoside triphosphates; dinucleotides corresponding to and encoded by the transcription start site of the leader; bacterial RNA polymerase complex; and tRNA specific for a specifier sequence located in the leader; and

b) one or more assay mixtures comprising: a potential inhibitor substance; a template

DNA that comprises: (i) a bacterial promoter, (ii) a leader of a T-box regulated gene, including a transcription start site, and (iii) a downstream polynucleotide of sufficient length for detection of a read-through mRNA product; divalent metal cations; nucleoside triphosphates; dinucleotides corresponding to and encoded by the transcription start site of the leader; bacterial RNA polymerase complex; and tRNA specific for a specifier sequence located in the leader.

19. The assay system recited in claim 18 wherein the divalent metal cation is Mg^{2+}.

20. The assay system recited in claim 18 wherein the nucleoside triphosphates are selected from the group consisting of adenosine triphosphate, guanosine triphosphate, cytosine triphosphate, and uridine triphosphate, and any combination of one or more of these.

21. The assay system recited in claim 18 wherein the dinucleotides are selected from the group consisting of ApA, ApC, ApU, ApG, GpA, GpC, GpU, GpG, CpA, CpC, CpU, CpG, UpA, UpC, UpU, and UpG.

22. The assay system recited in claim 18 wherein the bacterial promoter is selected from the group consisting of the *B. subtilis glyQS* promoter and the *B. subtilis rpsD* promoter.

23. The assay system recited in claim 18 wherein the downstream polynucleotide of sufficient length for detection of a read-through mRNA product comprises a polynucleotide which is from about 30 to 150 nucleotide residues in length.

24. The assay system recited in claim 18 wherein the tRNA specific for a specifier sequence located in the leader is *B. subtilis* $tRNA^{Gly}$.

25. The assay system recited in claim 18 wherein the RNA polymerase is from either *B. subtilis* or *Escherichia coli.*

26. The assay system recited in claim 18 wherein the leader comprises a polynucleotide which is a variant of a wild-type glycine synthetase leader from a Gram positive bacterial strain, wherein the variant comprises modifications to one or both of the wild-type specifier and wild-type terminator sequences.

27. The assay system recited in claim 18 wherein the tRNA specific for a specifier sequence located in the leader is a variant of a wild-type tRNA wherein any one or more of the wild-type anticodon sequence, the wild-type discriminator sequence, and the transcription start site is altered to complement with the leader sequence.

28. The assay system recited in claim 18 wherein the leader comprises a polynucleotide which is a variant of a wild-type glycine synthetase leader sequence from a Gram positive bacterial strain, wherein the variant comprises modifications to one or both of the wild-type specifier and wild-type terminator sequences, and wherein the tRNA specific for a specifier sequence located in the wild type leader is a variant of a wild-type tRNA wherein either or both of the wild-type anticodon sequence and the wild-type discriminator sequence are altered to complement with the variant leader sequence.

29. The assay system recited in claim 18 wherein the assay mixtures are *in vitro* halted-complex bacterial transcription assay systems.

30. An assay system for identifying inhibitors of transcription of bacterial T-box regulated genes, comprising:

two or more *in vitro* halted-complex bacterial transcription assay systems which comprise a template DNA comprising: (i) a bacterial promoter, (ii) a polynucleotide comprising a leader from the *B. subtilis* glyQS gene, including a transcription start site, and (iii) a downstream polynucleotide of sufficient length for detection of a read-through mRNA product; RNA polymerase, and uncharged *B.* subtilis $tRNA^{Gly}$, wherein at least one or more of said assay systems comprises a test substance, and wherein at least one or more of said assay systems lacks a test substance.

31. The assay system recited in claim 30 wherein the bacterial promoter is selected from the group consisting of the *B. subtilis glyQS* promoter and the *B. subtilis rpsD* promoter.

32. The assay system recited in claim 30 wherein the RNA polymerase is from either *B. subtilis* or *Escherichia coli.*

33. The assay system recited in claim 30 wherein the sequence of the polynucleotide comprising a portion of the *B. subtilis glyQS* leader is a variant of the wild-type *B. subtilis glyQS* leader sequence, wherein the variant comprises modifications to one or both of the wild-type specifier and wild-type terminator sequences.

34. The assay system recited in claim 30 wherein the uncharged *B. subtilis* $tRNA^{Gly}$ is a variant of a wild-type *B. subtilis* $tRNA^{Gly}$ wherein either or both the wild-type anticodon sequence and the wild-type discriminator sequence is altered to complement with a

polynucleotide encoding a variant *B. subtilis glyQS* leader.

35. A method for manufacturing an antibiotic agent, comprising:

Screening one or more test agents which are potential inhibitors of expression of T-box regulated genes by the following steps:

a) incubating one or more assay mixtures comprising: divalent metal cations; nucleoside triphosphates; dinucleotides; a template DNA that comprises: (i) a bacterial promoter, (ii) a leader of a T-box regulated gene, and (iii) a downstream portion; a bacterial RNA polymerase complex; and tRNA specific for a specifier sequence located in the leader portion of the template DNA; and

b) incubating a test substances with one or more assay mixtures comprising: a divalent metal cation; nucleoside triphosphates; dinucleotides; a template DNA comprising a bacterial promoter, the leader region of a T-box regulated gene, and a downstream portion; a bacterial RNA polymerase complex; and tRNA specific for a specifier sequence located in the leader portion of the template DNA; and

c) comparing the amount of the read-through mRNA product produced in step a) with the amount the read-through mRNA product produced in step b)

wherein a lesser amount of a read-through mRNA product determined for step b) in comparison with step a) indicates that said test agent inhibits transcription of T-box regulated genes and is an antibiotic agent.

Producing for use as antibiotic agents those test agents which inhibit production of a read-through mRNA product.